Appendix A: Visual Comparison of Lucas and Kanade Optical Flow (LK3D) and B-Splines Mutual Information Non-Rigid Registration (B-Splines).

The pipeline allows implementation of specific parts to differ, as long as their functionality is preserved. In this appendix we exemplify this freedom by further discussing the side-by-side results of two different deformation calculation methods: Lucas and Kanade Optical Flow (LK3D) and B-Splines Mutual Non-Rigid Registration method (B-Splines).

As mentioned in the main article, Optical Flow methods [1] result in a sequence of deformation velocity fields, where each vector estimates the direction and degree at which a local patch of tissue is deforming at a specific instant. Registration methods ([2] [3], on the other hand, result in deformation offset fields. In time-dependent deformation, the difference becomes apparent. Tracking a single point through the data domain over a single unit of time using velocity fields requires stepwise integration using time-interpolated deformation velocity data, whereas offset fields describe instant displacement over unit time, i.e. from one acquisition volume to the next. Deformation offset data can be used as deformation velocity data, provided that between acquisition steps the velocity data is not interpolated. However, this causes sudden velocity changes at acquisition step boundaries, which is detrimental to fluent visualization.

The LK3D [4] Optical Flow algorithm was applied to the cropped MRI dataset mentioned in section 1.1. Spatial and temporal image intensity gradients were calculated by convolution with a 4D Gaussian derivative kernel with $4.0mm^2$ x $4.0mm^2$ x $4.0mm^2$ x $0.09t^2$ variance, where *t* is the artificial unit of time between sequential MRI volumes. The algorithm itself is fully parameterized by a Gaussian weighting window with $0.64mm^2$ x $0.64mm^2$ x $0.64mm^2$ x $0.09t^2$ variance. The result is a sequence of 15 deformation data volumes of the same dimensions and resolution as the input data.

We also used the *Elastix* toolkit (http://elastix.isi.uu.nl/) for B-Splines of the same cropped MRI dataset. This resulted in a sequence of 14 deformation offset volumes of the same dimensions and resolution as the input volumes. The registration is based on a mutual information metric, and the deformation is parameterized by B-splines. The number of volumes for B-Splines is one less than the number of input volumes, because registration works on pairs of volumes.

The parameters we used for the deformation calculation methods were arrived at empirically. For LK3D, this meant minimizing variance for both intensity derivative calculation and the least squares estimation window, while still providing plausible results that allowed us to demonstrate our techniques. The B-Splines parameter file was assembled based on prior experience with the *Elastix* software and the data acquisition results.

The uncertainty field resulting from B-Splines is devoid of the clear structures that were present in LK3D. This is likely the cause of the uncertainty calculation method being better suited to the B-Splines deformation calculation results than to LK3D. This is related to the fact that LK3D produces deformation velocity data, whereas B-Splines produces deformation offset data. On the other hand, LK3D confidence is higher in stationary regions, e.g. bone and empty space. This may be caused by an effectively larger smoothing by LK3D as compared to B-Splines.

Comparison of the visual validation results for structures of known deformation (figures 7, 8 and 9 of the main article) allows us to get an initial, subjective estimate on which algorithm is more correct for our data. Figure 7 shows that for a simple rigid translation of a single dataset, the error in B-Splines is imperceptibly low, while LK3D shows definite distortion of the initial grid.

B-Splines also performs better than LK3D in tracking of the lens structure, as shown in figure 8. Although the shape of the lens is somewhat distorted, from the chosen perspective, the entire lens area remains covered by markers, strengthening its reliability as compared to LK3D.

The main problem with LK3D in the tracking of the blood vessel structure, i.e. the apparent ripping of tissue, is absent with B-Splines. This is, besides possible better performance in general, a side-effect from B-Splines' algorithmic design that does not allow tissue to tear. Also, the general position of the blood vessel is matched more closely by B-Splines than by LK3D (figure 9).

From these concededly simple validation tests, we can conclude that B-Splines performs better than LK3D, although we note that the tests did not include an example of diverging tissue.

Various differences between the LK3D and B-Splines results are observed in the region through which the optic nerve is dragged, see figure 3. Although, both algorithms indicate the general motion of the orbital fat in front of the optic nerve as partially forward and to the side, B-Splines lacks convergence of the soft tissue in the wake of the optic nerve. This indicates that tissue in the path of the optic nerve is moving along with it more as a solid, rubbery mass than LK3D that indicated a more or less liquid-like behavior of the orbital fat. The figure also shows the retained connectivity of the soft tissue where it is expected that the optic nerve would squeeze it from between itself and the medial rectus muscle. As mentioned in the previous paragraph, this is an algorithmic effect of B-Splines.

Differences in orbital motility between LK3D and B-Splines are also observed between the eyeball and the medial rectus muscle as the muscle rolls up onto the globe during abduction, see figure 6 (top). Although the amount of stretching of the soft tissue near the rectus muscle is larger for B-Splines than for LK3D, the same problems as described before arise; not enough stretching and tissue remains where it should be evacuated. The last problem has the same cause as in the previous example: B-Splines does not allow tissue to rip.

Finally, we show the difference between LK3D and B-Splines in the region where the lateral rectus muscle rolls off from the eyeball during abduction. B-Splines present a more plausible picture, showing a slight amount of compression, in addition to the generally correct motion of markers rotating with the eyeball (figure 6, bottom).

Our experience with two different deformation calculation methods, i.e. Lucas and Kanade Optical Flow and mutual information-based non-rigid registration with the deformation parameterized by B-splines, has brought several issues to our attention. First, neither of the methods necessarily results in physically correct deformation data. This can be seen in the LK3D results by the disconnection of rigid structures, shown in Figure 9. B-Splines retains connectivity in the deformation of the blood vessel structure, but by design is unable to show disconnection of tissue regions as should happen as, for instance, the optic nerve slides through the fat or one of eye muscles rolls up onto the globe, evacuating the fat between, see figures 3 and 6. Secondly, during experimentation with the image derivative and LK3D parameters, we found that only a small range of variances give plausible deformation results,

which vary from erratic to feature-less in a short variance span. Also, kernel widths should be such that the Gaussian contributions near the kernel edges are negligible, thereby reducing sudden changes in the deformation field that lead to strongly diverging and converging regions. B-Splines, on the other hand, is less sensitive to small parameter deviations. Together, these factors suggest that B-Splines is the better choice for deformation calculation from a sequence of MRI volumes, although we cannot exclude LK3D as the method of choice after proper calibration.

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